Augmentation of the Inflammatory Reaction by Activity of the Central Nervous System

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As an aspect of the continuing study in this laboratory of the participation of the nervous system in integrating appropriate and inappropriate adaptive reactions,[1] the inflammatory response has been of prime interest.

It was a Galenic dictum that any part of the body may influence any other part through neural connections. In the 18th century, Cullen, Pinel, and Baglivi held the view that disorders of the nervous system underlay most disease processes. This emphasis gave rise to the school of "systematic correlative neuropathologists," which dominated French concepts of disease in the first half of the 19th century.[2] In the latter half of the 19th century growing emphasis on localized cellular alterations overshadowed the earlier interest in generalized reactions.[3]

Cohnheim, in 1882,[4] inferred that vasomotor nerves played no part in inflammation, but by 1909 it was established (by the work of Samuel, Meltzer, and Meltzer, and of Roger, Adami, and others), as cited by Florey,[5] that interruption of sympathetic vasoconstrictor nerves led to vasodilatation and enhanced inflammatory reactions following noxious stimulation. On the other hand, interruption of vasodilator fibers resulted in constriction and diminished inflammation subsequent to injury. The latter procedure delayed elimination of injected micro-organisms and slowed recovery from injury.

Bruce, in 1910,[6] was among the first to suggest that an axon reflex was involved in the vasodilatation of inflammation. In the 1920's and 1930's several investigators studied the participation of the nervous system in the inflammatory process.[7-9] Sir Thomas Lewis[9] analyzed the "triple response" of the skin to injury and showed that a red central area surrounded by a brighter red flare appeared upon injuring the normal skin, but that if the sensory nerves to the skin had been cut five to seven days before the injury, only the central red area developed. If the injury occurred before the peripheral nerve fibers had degenerated, the surrounding flare was still evident. He regarded the central red area as being due to dilatation of venules and capillaries that had been acted upon directly by a chemical substance liberated by the injury tissue. This substance, termed H substance because of the similarity of its effects to those of histamine, was thought to stimulate afferent nerve endings in the injured area. In this way, an axon reflex was initiated that dilated arterioles outside the injured area and resulted in "flare." Recently, potent pharmacodynamic agents have been demonstrated to participate in inflammation.[10-17]

Previous studies from this laboratory have demonstrated that many of the organs

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of the body become more readily damaged during or after periods perceived as threatening: Vasodilatation, edema, diapedesis, hemorrhage, erosion, increased friability of tissue, lowered pain threshold, and impaired organ function have been observed in the skin,[18] nose,[19] airways,[19] stomach,[20] colon,[21] bladder,[22,23] vagina,[24] and the subcutaneous tissue of the scalp.[25] These changes could be induced rapidly by appropriate alteration of the environment, as by interviews which augmented or decreased the perception of threat.

The following section is a report concerning the pertinence of vasomotor influences and certain proteolytic enzymes and polypeptides released or accumulated not only when lower levels of the nervous system but also when the highest levels are involved in adaptive responses. The participation of various levels of activity of the nervous system in inflammation was analyzed through study of modifications of the inflammatory response to noxious stimulation associated with the axon reflex, the thermoregulatory reflexes, and suggestions during hypnosis.

I. Demonstration That the Inflammatory Response Can Be Modified by Neural Activity Integrated at the Segmental Level: the Axon Reflex

A. The pain threshold is lowered in the flare zone of the axon reflex.

Bilisoly, Goodell, and Wolff [26] observed that the pain threshold in the flare area of the axon reflex was lowered, as measured by the hair-pull method, by thermal dolorimetry, and by von Frey hairs. The axon reflex was studied as an example of the simplest integrative pattern that could be relevant to inflammation. Axon reflexes were induced on the volar surface of the arms by intradermal injection of 0.05 cc. of histamine phosphate (1:1,000) in six subjects. The lowered pain thresholds in the flare zones were demonstrated repeatedly in each subject.

B. The response to injury is heightened in zones of vasodilatation.

Bilisoly, Goodell, and Wolff [26] also used the axon-reflex flare to illuminate the relation between vasodilatation and heightened tissue vulnerability. Since the pain threshold was lowered during the period of active vasodilatation, it was suggested that an added standard noxious stimulation would have a more damaging effect within such an area than in control areas. Hence, axon-reflex flares were induced on the volar surface of one arm, and the other arm served as a control. It was then shown that, as the result of exposure to similar intensities of noxious thermal or chemical stimulation, there was significantly more skin damage in the zone of flare on the one arm than in comparable areas on the control arm. These differences were most striking 48 hours after thermal injuries.

C. Protease and bradykinin-type polypeptides are increased in amount in subcutaneous perfusate collected from the flare zone of the axon reflex.

Previous studies of blister fluid by Armstrong, Keele, and associates [15] and of the subcutaneous fluid collected from the scalp during vascular headache of the migraine type by Ostfeld et al.[25] indicated that this tissue fluid contained increased amounts of substances resembling polypeptides of the bradykinin type, as defined by Rocha e Silva et al.[27] Hence experiments were devised that permitted the collection of a subcutaneous perfusate from the zone of axon-reflex flare induced by injuring the skin by intracutaneous histamine phosphate at least 4 cm. distal to the site of fluid collection. In other instances the skin was damaged by heat or by cooling with ice.

Subjects and Method:

The subjects studied were two healthy adults and three patients: One man had had a gunshot injury of the brachial plexus, with absence of sensation in the region stimulated and perfused; one woman had absence of sympathetic innervation of the forearm, as a sequel of sympathectomy, and a second woman had "cold allergy"; i.e., she readily developed urticaria in response to cold stimulation.

The method of perfusing the subcutaneous space is a modification of that of Fox and Hilton.[28]

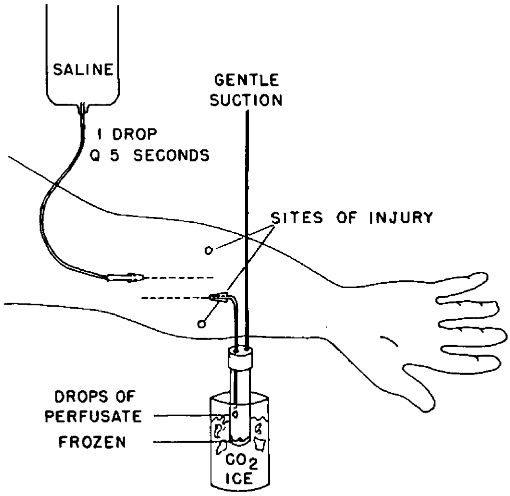


Fig. 1.—The arm prepared for perfusion and immediate freezing of perfusate as collected. Two No. 20, 2-in.-long perforate needles were inserted into the subcutaneous spaces parallel to each other and at a distance of approximately 1.5 cm. apart. This method is an adaptation of that of Fox and Hilton.

Figure 1 shows the method of inflow and outflow, using perforate needles. These needles were No. 20 steel hypodermic needles with eight perforations in the shafts.* The needles were inserted into the subcutaneous space of the volar surface of the forearm parallel to each other and approximately 1.5 cm. apart. The rate of inflow of the perfusion fluid (isotonic saline) was 0.5 cc. a minute. The perfusate was collected in siliconized tubes and was immediately assayed or frozen in CO_2 snow to prevent alteration and subsequently assayed when convenient. The perfusate was assayed by the conventional smooth muscle (rat uterus[27] and rat duodenum[29,30]) methods, illustrated in Figure 2. After the collection of four to six control specimens of perfusate, at five-minute intervals, two intracutaneous injections of histamine were made at a distance of approximately 4 cm. on either side of the collection site. Five-minute samples of the perfusate were collected during 30 to 60 minutes thereafter. Aliquots (0.2 cc.) of the samples were added to the chamber (Fig. 2), and the amplitude of contraction (rat uterus) or relaxation (rat duodenum) induced was expressed as the amount of bradykinin standard required to induce similar contraction or relaxation. Twenty perfusion experiments were performed. Perfusates from the two intact subjects were assayed with the rat uterus method in 10 experiments, with the rat duodenum method in 3 experiments, with the guinea pig ileum method in 2 experiments, and, for protease activity, with a titrimetric method in 2 experiments. In the last method benzoyl arginine ethyl ester (0.01 M) was incubated with perfusate at 37 C in the presence of "tris" buffer (0.25 M, pH 9). Esterase activity was determined by titration of free carboxyl groups formed. Esterase activity determined with this substrate may be used as a measure of protease activity.[31,32] The perfusates from the three patients were assayed with the rat uterus method.

* The perforate needles were especially made by Becton, Dickinson & Co., Rutherford, N. J.

APPARATUS FOR RAT UTERUS BIOASSAY

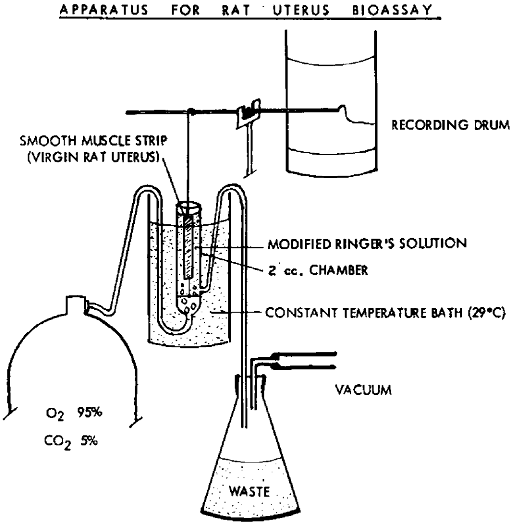


Fig. 2.—The arrangement of apparatus for the method of rat uterus (or rat duodenum) bioassay of perfusate collected according to the method illustrated in Figure 1.

Results:

Control samples of perfusate collected during intervals as long as 60 minutes resulted in contractions equivalent to approximately equal small amounts of bradykinin, after initially higher amounts in the first one or two specimens collected after insertion of the perfusion needles. The presence of this bradykinin-like substance in the control specimens could have resulted in part from trauma secondary to the perfusion procedures and in part from activation of proteolytic enzymes by dilution of serum proteins in the extracellular fluid with saline.

The axon-reflex flares began soon after the skin was injured by the injections of histamine, the flares gradually increasing in area and redness during 15 to 30 minutes. During the first few minutes after the injury, the perfusate contained augmented

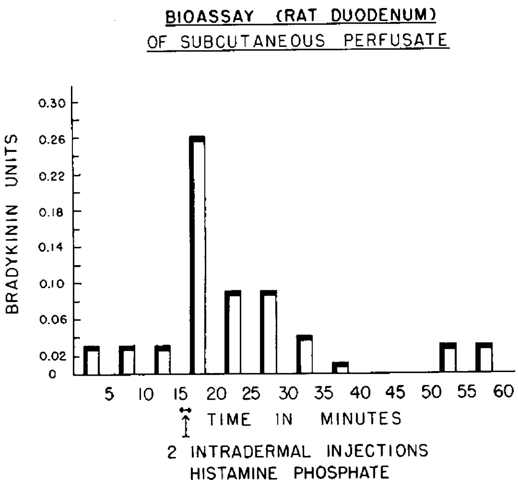


Fig. 3.—Bioassay (rat duodenum) of subcutaneous perfusate collected before and during axon-reflex flare. The axon reflex was induced in two areas by intracutaneous injection of 0.05 cc. of histamine phosphate (1:1,000) at distances of approximately 4 cm. on either side of the perfusion needles (Fig. 1). The duodenum was suspended in de Jalon's solution at 29 C, containing atropine (1×10^{-4} gm/L.), tripelennamine (Pyribenzamine) (1×10^{-4} gm/L.), and BOL (1×10^{-4} gm/L.).

amounts of bradykinin-like substance, as assayed with the rat duodenum (Fig. 3). The fluid collected in the second and third five-minute periods after noxious stimulation also resulted in larger contractions than in the control periods. The flare, however, persisted for a longer period than the increased activity in the perfusate.

The subject with brachial plexus injuries and absence of sensation in the region stimulated and perfused had no flare and no itching following the histamine injection. Also, the perfusate contained no increase in the amount of bradykinin-like material after histamine injection. On the other hand, in the subject who had been deprived of sympathetic innervation only, flare developed after histamine injection, as in the intact subjects, and bradykinin-like polypeptides in the perfusate were increased threefold. This observation will be discussed in more detail later.

Figure 4 shows the results of bioassay of subcutaneous perfusate with the rat uterus. The time course of increased activity differed from that observed with the rat duodenum method. This difference suggests that several substances are present in increased amounts at different times during the axon-reflex flare, since some agents, for example bradykinin, contract the rat uterus but relax the rat duodenum, while others, for example potassium, contract both tissues. Mixtures of such agents may be

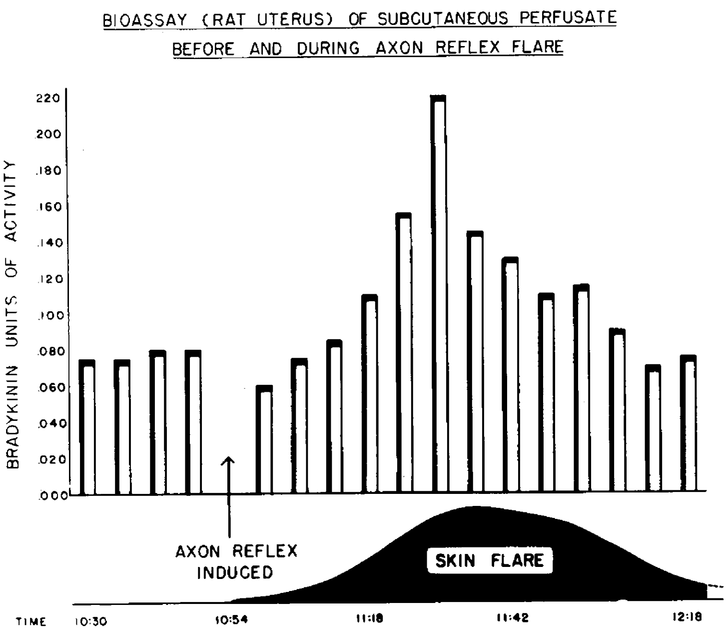


Fig. 4.—Bioassay (rat uterus) of subcutaneous perfusate collected before and during axon-reflex flare. The flare was induced as in Figure 3. The uterus was suspended in de Jalon's solution to which atropine (1×10^{-4} gm/L.), and BOL (1×10^{-4} gm/L.) had been added.

additive in their effects on one preparation but antagonistic on the other.

The forearm of the patient with the "cold allergy" was perfused, as described above. After the collection of several six-minute control specimens of perfusate, an extensive urticarial reaction and flare were induced by the application of cold to the area on either side of the perfusion needles. Bioassay revealed a fivefold increase in bradykinin-like polypeptides.

The properties of the agent responsible for the augmented rat uterus contractions were further defined by study of inhibitors of serotonin and acetylcholine. The rat uterus was suspended in the 2 cc. bath containing de Jalon's solution to which the bromine derivative of lysergic acid diethylamide (1×10^{-4} gm/L.) and atropine (1×10^{-4} gm/L.) had been added. These substances did not inhibit either the contractions induced by control specimens or the increase observed during the axon-reflex flare, indicating that the substance responsible for the increased contractions was not principally serotonin or acetylcholine.

The activity of the perfusate, as defined by its capacity to induce contractions of the rat uterus, was unstable at room temperature. The activity of frozen samples collected in the first five minutes after noxious stimulation increased if the fluid was allowed to stand for three minutes at room temperature but decreased when allowed to stand longer periods; after 10-15 minutes no activity could be demonstrated. The activity of control specimens and that of the specimens gathered subsequent to the first five minutes after noxious stimulation diminished slightly on standing three minutes at room temperature and continued to decrease thereafter. It was postulated that this rapid destruction resulted from peptidase activity in the extracellular fluid. Accordingly, the samples were heated for four minutes in a boiling water bath to destroy such enzymes, and it was found that activity of the perfusate was thus stabilized. Further evidence that the principal agent was a bradykinin-like polypeptide was furnished by the observation that brief (three-minute) incubation with chymotrypsin reduced the activity of the perfusate collected during flare to less than the minimally observable amount that could be detected by the method. This enzyme is known to inactivate polypeptides of the bradykinin-type.[33]

Preliminary studies of the perfusate collected from two subjects indicated the presence of increased amounts of protease, as assayed by the chemical method, using benzoyl arginine ethyl ester as substrate, in the five-minute specimens collected immediately after noxious stimulation, as compared with "control" specimens or the perfusate collected more than five minutes after noxious stimulation. In this laboratory there has been good agreement between protease activity, as determined with this substrate, and "bradykinin-forming" activity, as measured by bioassay with smooth muscle.

Comment:

Since histamine can be released by proteolytic enzymes,[34] and since bradykinin formation is dependent upon such enzymes, it is conceivable that histamine is liberated under conditions associated with the formation of polypeptides. Indeed, other recent experiments in this laboratory indicate that histamine liberation may be a significant factor in the axon and Louvain reflexes.[25] Other agents probably also participate. Serotonin might be released in those reactions in which there is platelet accumulation and breakdown, and possibly escape of serotonin from minute blood vessels into the tissues. Benjamin [35] has recently suggested that noxious stimulation releases intracellular potassium which then activates pain endings. Holton [36] has demonstrated increased adenosinetriphosphate (ATP) content of rabbit ear perfusate following antidromic stimulation. Kunkle [37] has shown that acetylcholine is elevated in cerebrospinal fluid during some vascular headaches of the migraine type. However, since ATP

Properties of a Substance in Subcutaneous Perfusate Present in Increased Amounts During Axon-Reflex Flare

1. Induces itching and dilatation of small vessels and lowers pain thresholds when reinjected intradermally.
2. Increases vessel permeability (outflow volume of perfusate increases two- to threefold during flare).
3. Contracts isolated rat uterus and guinea pig ileum and relaxes isolated rat duodenum.
4. Enhanced smooth muscle contractions are not inhibited by antihistamines, the bromine derivative of lysergic acid diethylamide (BOL), or atropine in amounts that inhibit histamine, serotonin, and acetylcholine, respectively. Thus, none of these is the principal active agent, although they or others may also participate.
5. Rapidly loses its capacity to induce contractions of smooth muscle on standing at room temperature (in 10 to 20 minutes).
6. Its activity is stabilized by four minutes of heating in a boiling water bath.
7. The activity of the heat-stabilized substance is destroyed after brief (15-minutes) incubation with chymotrypsin; i.e., the active agent is a peptide.

and acetylcholine are usually rapidly destroyed in tissue, their significance in sustained inflammatory reactions is difficult to evaluate.

It has been shown that when mixtures containing bradykinin prepared by incubation of trypsin with pseudoglobulin were injected into the skin, they induced burning pain and itching sensations.[38-40] The accompanying Table lists these and other properties of the perfusate supporting the view that the principal active agent liberated into the perfusate is a bradykinin-type polypeptide.

At least three possibilities concerning the origin of protease and polypeptides are compatible with the data here presented. The first is based on the fact that blood itself contains an enzyme (plasmin) that can form bradykinin-like polypeptides.[41] According to the views of Lewis,[42] the entrance of this enzyme system into the interstitial spaces as a consequence of vasodilatation induced by some unknown mechanism could result secondarily in the formation of vasodilator polypeptides (plasma kinins).

The second possibility is that neurogenic influences release metabolites that convert plasminogen already present in extracellular fluid to the active proteolytic enzyme, plasmin, which then results in polypeptide formation.

A third, and to us attractive, possibility is that a neurogenic humoral agent, such as acetylcholine, alters membrane permeability of many varieties of cells to release intracellular proteolytic enzyme, which then acts on protein in extracellular fluid to produce polypeptides of the bradykinin type, as well as other potent agents, such as histamine and serotonin.

Hilton and Lewis,[43] analyzing venous perfusate from the skin of the cat, failed to observe bradykinin activity following antidromic stimulation of the saphenous nerve. However, the degree of vasodilatation in these circumstances was slight, and an associated small increase in bradykinin might conceivably have been beyond the limitations of the method to detect.

Protease activity has long been held to be relevant to inflammation,[44] and Ungar [45] recently has traced the evolution of the concept that proteolytic-enzyme activation is a primary feature of the antigen-antibody reaction. Also, Beraldo, in 1950,[46] demonstrated that bradykinin is formed during anaphylactic shock. Thus, on the basis of these latter studies and those reported in the present communication, it appears that bradykinin formation is a common biochemical link between nervous system activity and the antigen-antibody reaction.

II. Demonstration That the Inflammatory Response Can Be Modified by Neural Activity Integrated at the Brain Stem and Hypothalamic Levels: Thermoregulatory Reflexes

A. Thermoregulatory reflex vasodilatation is accompanied by lowered pain threshold.

The vasomotor reactions in the skin as part of the body's thermoregulatory devices can be dramatically demonstrated by the study of the nonimmersed surface of a subject partially immersed in hot water. Under these circumstances the skin of the nonimmersed portion becomes flushed, the temperature elevated, and sweating aug-

mented. Graham et al.[47] showed that during the initial flush there was a transient lowering of the pain threshold in the skin, as measured by the thermal radiation technique. Bilisoly et al.[26] also induced reflex vasodilatation in the skin of the arms by immersing the subject's feet and legs in hot water. By using the point at which pulling a hair first caused pain as a pain-threshold measurement, they demonstrated a transient lowered pain threshold during the initial flush. This lowered threshold was not dependent upon an elevated skin temperature per se.

B. Thermoregulatory reflexes can modify the vaosdilatation accompanying the axon reflex.

Two experiments were performed in order to ascertain whether the magnitude of the flare of the axon reflex could be modified by superimposing the effects of the thermoregulatory reflexes just described. Two of us served as subjects. First, the unclothed subject was chilled in moving air at a room temperature of approximately 14 C. After 30 minutes of chilling, the skin of one forearm was injured by intracutaneous injection of histamine phosphate (0.05 cc. of 1:1,000 solution). The extent of axon-reflex flare was outlined in ink and a tracing made on thin paper for comparison with the flares (*a*) resulting from a similar injury an hour later, when the subject was again comfortably warm, and with the flares (*b*) induced after the subject had been heated by immersion of the feet and legs in hot water. The flares induced when the subject was chilled were invariably far smaller in area than those in control conditions or during heating (Fig. 5).

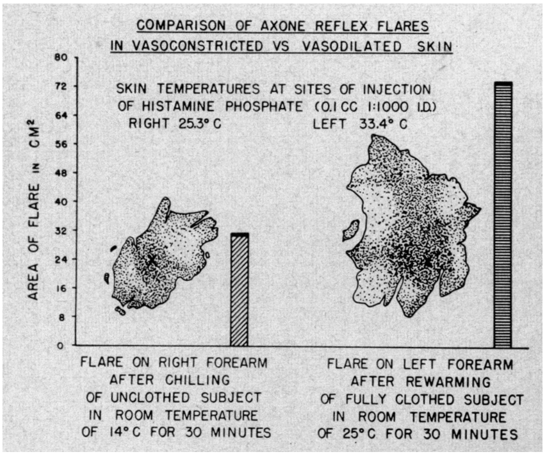


Fig. 5.—Comparison of axon-reflex flares in the chilled vs. the normal, warm subject.

C. The magnitude of inflammatory reaction and tissue damage in response to noxious stimulation is enhanced during thermoregulatory reflex vasodilatation.

In the second series, of five experiments on three healthy subjects, the skin of the forearms was injured by a standard thermal stimulus (500/mc/sec/sq. cm. for three seconds) applied to one arm before the subject was immersed (control, "comfortably warm" conditions) and to the other arm when the subject was being heated by immersion of the feet and legs in hot (42 C) water and just as the skin temperature of the forearm was beginning to rise. If the skin temperature had become elevated, the skin was gently cooled locally with a forced draft of air to the temperature of the control arm at the time it had been injured. Skin temperatures were recorded by a multichannel continuous recorder. The

Fig. 6.—Photographs of the results of burns resulting from application of similar noxious stimulation (500/mc/sq. cm/sec. for three seconds) to the right arm just before, and to the left arm during, immersion of the feet and legs in hot water. The burns on the left arm were made in the initial phase of vasodilatation and can be seen to be more extensive than on the right, control arm.

magnitude of the inflammatory reaction was estimated and photographed each day during the next 12 days. In all three subjects, and in all experiments, the injuries sustained on the arms noxiously stimulated during immersion of the feet and legs were significantly greater than those on the control arms; an example is illustrated in Figure 6. This picture was taken three days after the skin had been burned. The skin temperature just before the skin was burned was 34.0 C (93.2 F) on the right control arm and 33.7 C (92.6 F) on the left, cooled, though actively vasodilating, arm.

Comment:

The experiments with chilling the body at low room temperature, and with raising body temperature by immersion in hot water, implicating the thermoregulatory centers, demonstrate that activity within the central nervous system integrated at the brain stem and hypothalamic levels can modify the effects of the axon reflex and the inflammatory reaction to noxious stimulation. Fox and Hilton [28] demonstrated that the flush induced on the forearm by immersing the rest of the body in hot water is accompanied by an increase of bradykinin-like material in the perfused subcutaneous spaces. It may thus be inferred that central neural activity can lead to increased amounts of proteolytic enzymes and bradykinin-like polypeptides in the peripheral tissues in which there is thermoregulatory reflex vasodilatation.

D. Activation of sweat glands is not essential to bradykinin formation during neurogenic vasodilatation in the skin.

Fox and Hilton [28] inferred that heightened sweat-gland activity is responsible for the release of bradykinin-forming enzymes during vasodilatation induced by indirect heating. Experiments were undertaken to clarify whether or not sweat-gland activation is indeed essential to the increased production of bradykinin-like polypeptides during axon reflex, by study of a subject deprived of sympathetic innervation in the region examined. The patient, one with Raynaud's syndrome, 12 years earlier had undergone bilateral upper thoracic sympathectomy and bilateral anterior rhizotomy of the first thoracic nerve. The sympathetic chain was divided below the third ganglion, and the second and third intercostal nerves were resected. The stellate, second, and third ganglia and the central attachment of the first thoracic root were left intact. Also, the first thoracic anterior root was divided bilaterally, leaving the posterior roots intact.

The left forearm was perfused as described above (Fig. 1). Histamine phosphate (0.05 cc. of 1:1,000 solution) was injected intradermally approximately 4 cm. on either side of the perfusion site to injure the skin and induce an axon reflex. A "flare" developed that was similar to that observed in intact subjects. However, there was no change in the electrical resistance of the implicated skin and no evidence of sweating with the starch-iodine method. Nevertheless, a threefold increase in bradykinin-like substance was found in the perfusate collected during the flares.

Comment:

These observations argue against the suggestion of Fox and Hilton [28] that activation of sweat glands is a required step in the release of bradykinin-forming enzyme during neurogenic vasodilatation and heightened metabolism in the subcutaneous skin and tissues, but do not deny that the sweat glands when secreting may contribute to formation of bradykinin-like substance. Indeed, there is nothing to indicate that the release of intracellular protease during heightened cellular activity is limited to specific cells. Chapman and Wolff [48] demonstrated increased amounts of kinin-forming enzymes in cerebrospinal fluid following noxious stimulation. Ansell and Richter [49] demonstrated a neutral proteinase in whole-brain slices, and Ungar [50] showed that protease activity of whole-brain slices is increased following stimulation of sciatic nerve.

III. Demonstration That Central Nervous System Activity Integrated at the Highest Level Modifies the Inflammatory Reaction in the Periphery

A. Spontaneous urticaria may occur in situations perceived as threatening.

Graham, studying patients with urticaria about 10 years ago,[51] showed in situations perceived as threatening, or during the discussion of them, that the tone of the minute vessels in the skin was reduced and permeability increased. Under these circumstances heretofore non-noxious stimuli, both mechanical and chemical, became capable of inducing flush, itching, and urticaria, and in some instances these latter phenomena occurred spontaneously.

Although careful descriptions of methods and results are rare, it has long been alleged that lesions in the skin can be induced by suggestion during hypnosis. A recent and better-documented instance is that of Ullman, who induced erythema with blister formation that was noted at the end of an hour, and was fully developed approximately four hours later.[52]

In this laboratory it was possible during hypnosis to demonstrate the occurrence of erythema and whealing in response to the repeated application of a metal rod at room temperature to the skin of the forearm, accompanied by the suggestion that the rod was hot and would burn the skin.

B. Suggestion during hypnosis may modify the magnitude of vascular response and the degree of tissue damage in the skin following noxious stimulation.

The above observation that erythema could be induced by suggestion was further evidence that activities of the highest integrative levels of the nervous system could modify the magnitude of inflammatory reactions in the periphery. Therefore, experiments were designed to demonstrate that the degree of inflammatory response and tissue damage to a standard noxious stimulation could be augmented or diminished by activity of the central nervous system integrated at the highest levels. As an aid in manipulating these activities by the suggestion of relevant symbols, the hypnotic state was induced. It was, however, recognized that the hypnotic state per se is not essential to the effects associated with suggestion, symbols, and memories.

Method:

Thirteen adult men and women served as subjects. Three of these were patients who had spontaneously occurring urticaria. The remainder were healthy and free of skin disorders.

Experiments were conducted in a quiet, semidarkened, comfortably cool room. Thermocouples were attached to the subject's arms and a series of control temperatures recorded by means of a multichannel recording instrument. Finger cuffs for recording of the plethysmograms were attached to the middle finger of each hand and control recordings made.

The subject was hypnotized in the conventional manner. As soon as a state of moderate to deep hypnosis had been established, it was suggested either that one arm was "normal" or that it was "numb," "wooden," and devoid of sensation ("anesthetic"). It was then suggested that the other arm was painful, burning, damaged, and exceedingly sensitive, i.e., "vulnerable." Furthermore, it was suggested that severe injury to this "vulnerable" arm, which would cause even greater pain and damage, was about to occur. The "vulnerable" arm was then exposed on three spots, blackened with India ink, to standard noxious stimulation (500/mc/sq. cm/sec. for three seconds). After an interval of 15 to 30 minutes, during which hypnosis was continued, suggestions that the other arm was either "anesthetic" or "normal" were repeated and reinforced. It was then similarly exposed on three spots to the standard noxious stimulation. In some experiments the order of exposing the "vulnerable" and "anesthetic" arm to noxious stimulation was reversed. Also, the right arm was suggested to be "vulnerable" in some experiments, the left in others. In some experiments the suggestions of "vulnerability" for one arm and "anesthesia" for the other were made simultaneously, and the noxious stimulation was applied during the same interval alternately on the two arms.

The inflammatory reaction and tissue damage were assessed by observations and measurements of area, intensity, and duration of erythema, edema, blister formation, necrosis, and, when present, residual scar formation. Colored photographs were made approximately 20 minutes after the end of an experiment and at 24-hour intervals for about 2 weeks.

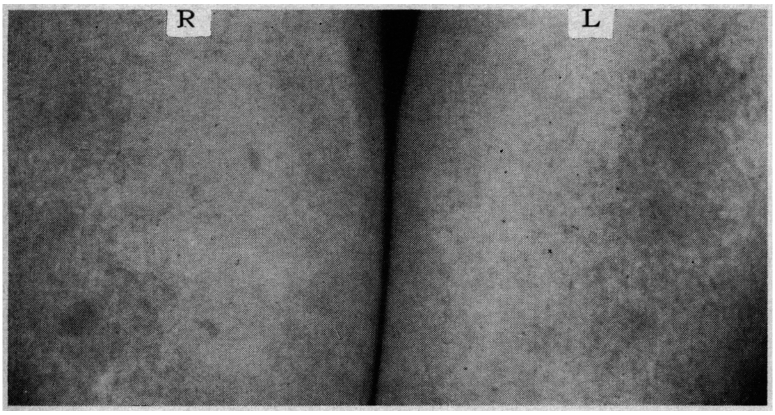


Fig. 7.—Photograph of the arms of Subject A. made approximately half an hour after noxious thermal stimulation during hypnosis. The right arm as suggested to be "vulnerable," the left arm "anesthetic." The burns were made in quick alternation from one arm to the other within a period of 30 seconds. Note the more extensive erythematous reaction surrounding the burns on the "vulnerable" (right) arm.

In one subject the subcutaneous spaces in the zones of axon-reflex flares induced by the noxious stimulation were perfused. The method of perfusing the subcutaneous space is a modification of that of Fox and Hilton.[28] Figure 12 shows the method of inflow and outflow, using perforate needles.

Results:

The 13 subjects were hypnotized in 40 experiments. During 12 of these experiments increased pain and damage were suggested in one arm, and suggestions that the arm on the opposite side was normally sensitive were made. The inflammatory reaction and tissue damage were greater on the side of suggested "vulnerability" in 9 of this series of 12 experiments; in 1 experiment increased damage was observed on the side of suggested normal sensitivity, and in 2 experiments no difference was noted. In 27 experiments it was suggested that one arm was "vulnerable" and that the other arm was "anesthetic." In one experiment greater damage occurred in the arm of suggested "anesthesia." In six experiments no difference was observed in the two arms. However, in 20 of this series of 27 experiments the inflammatory reaction was greater on the side of suggested pain and damage. In one experiment it was suggested that one arm was anesthetic, the other normally sensitive. The reaction was far greater on the normal side. Although the contrast was greatest in the magnitude of reaction between suggested "anesthesia" and suggested "vulnerability," it was also evident that the effect of suggested "anesthesia" was to suppress the inflammatory reaction, as contrasted with the effect of suggestion of normality. In Figures 7, 8, 9, and 10 are

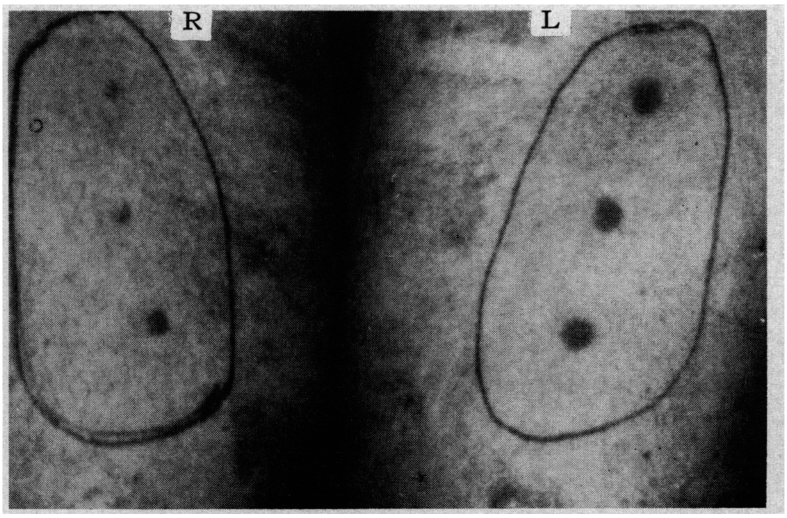


Fig. 8.—The arms of Subject A. photographed four days later than the photographs of Figure 7. Note the greater tissue damage on the "vulnerable," right arm, as compared with that on the "anesthetic," left arm, resulting from identical intensities and durations of noxious thermal stimulation.

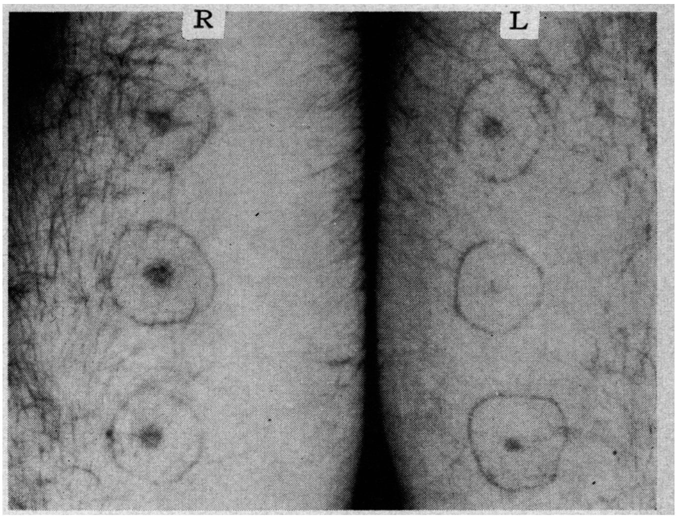
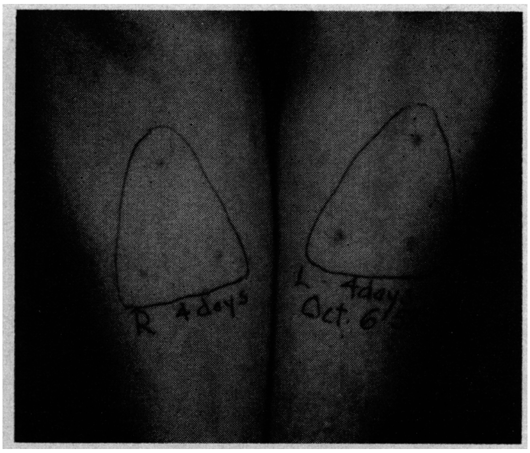


Fig. 9.—Photograph of the arms of Subject B., made three days after noxious thermal stimulation during hypnosis. The burns were made in quick alternation from one arm to the other while the left arm was being suggested as "vulnerable" and the right arm as "anesthetic." Note the greater tissue damage on the "vulnerable," left arm, as compared with that on the "anesthetic," right arm, resulting from identical intensities and duration of noxious thermal stimulation.

shown the greater damage in the vulnerable arms and the less severe damage in the "anesthetic" arms after identical thermal stimulation of the two limbs. One patient who complained of spontaneously occurring urticaria developed urticarial reactions in other parts of her "vulnerable" arm following noxious stimulation by heat (500/mc/sq. cm/sec. for three seconds).

Fig. 10.—Photograph of the arms of Subject C. four days after noxious thermal stimulation during hypnosis. While the right arm was suggested to be "anesthetic," three thermal burns were made. Hypnosis was continued, and half an hour later suggestions of "vulnerability," i.e., that the left arm had already been damaged and was painful and sensitive, were made; and it was repeated several times that it would be burned and damaged again. During these suggestions noxious thermal stimulation was applied three times. Note the greater burns on the left, the "vulnerable," arm.



Urticaria was not observed on the "anesthetic" arm at this time.

In two experiments in two subjects, two exposures of 500 mc. and two exposures of 410 mc. were made on each arm. The less intense stimulation was used in order to ascertain whether the effects of highest level functions would be more conspicuous when the damaging stimulus was less intense. It was shown that when anesthesia was suggested in the one subject no visible damage could be perceived 18 hours after the stimulation with 410 mc., whereas when

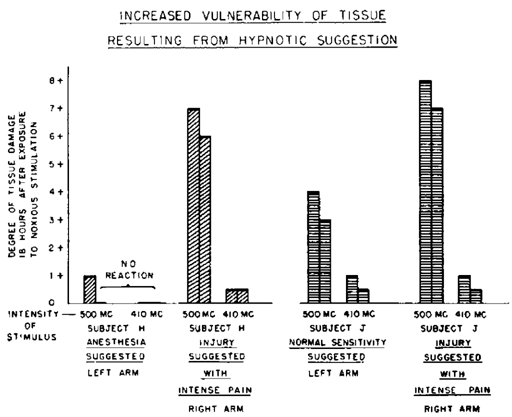


Fig. 11.—Diagrammatic representation of increased vulnerability of tissue induced during hypnotic suggestion in two subjects, H and J. Two intensities of thermal stimulation were used, and the effects of suggested anesthesia compared with those of "vulnerability" in Subject H, and the effects of suggested normal sensitivity compared with those of "vulnerability" in Subject J.

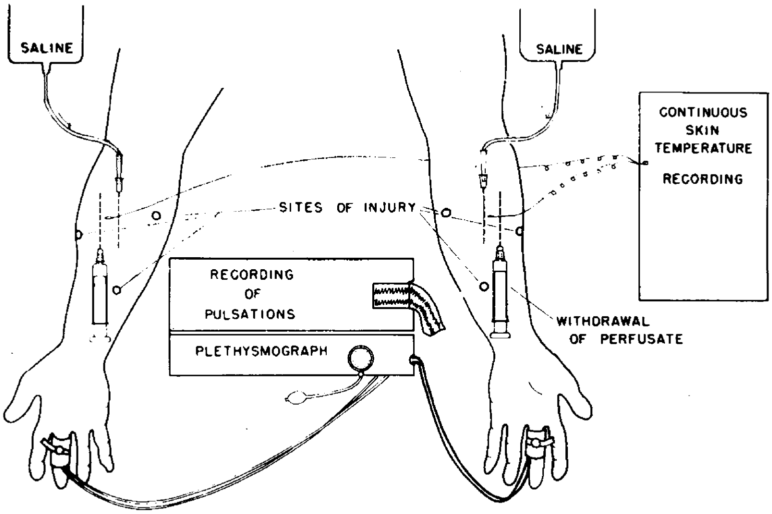


Fig. 12.—Arrangement of apparatus and recording devices for perfusion of two arms, collection of perfusate, recording of finger pulsations, and measurement and recording of skin temperature, with the sites of injury indicated. Four No. 20, 2-in.-long, perforate needles were inserted into the subcutaneous spaces. Thermocouples for skin-temperature measurement were attached to the skin at a distance of 2 cm. from the site of injury.

injury with intense pain was suggested, this less intense stimulus produced readily detectable lesions.

In the second subject the difference in reaction between the arm with normal sensitivity and the one with suggested injury and intense pain was less striking, but the inflammatory response to the more intense stimulation was conspicuous (Fig. 11) on the side of suggested "vulnerability."

Experiments were then designed to ascertain the nature of the relevant tissue responses that could explain the greater amount of damage in the "vulnerable" arms. Figure 12 shows the two arms prepared for skin temperature, for finger plethysmographic recordings, and for the collection of perfusate. These observations were made before and during the period of hypnosis, both before and after the application of noxious stimuli. In this series no significant differences in the amplitude of finger pulsations, in the skin temperature of the arms, or in the bradykinin content of the perfusates could be observed between the "anesthetic" and the "vulnerable" arm before the damaging stimulus was applied, although other experiments in this laboratory indicate that such differences may be induced during suggestion alone. Under the given experimental circumstances reported here, in response to noxious stimulation there was a greater and longer-sustained rise in the amplitude of the finger pulsations and in the skin temperature of the "vulnerable" arm (Figs. 13 and 14), as well as a greater increase of bradykinin content

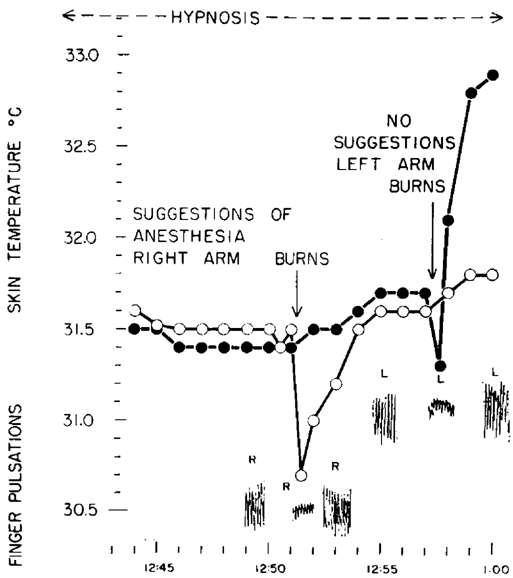


Fig. 13.—Measurements during hypnosis of the skin temperature and amplitude of finger pulsations of the right, "anesthetic" arm and of the left arm, suggested to be normally sensitive. Note the larger initial vasoconstriction, as indicated by fall in skin temperature in the arm suggested to be anesthetic (right) and the markedly reduced subsequent vasodilatation as compared with the reaction to the burns on the normally sensitive (left) side. The absence of, or the minimal, vasodilatation after injury in the arm suggested to be "anesthetic" affords a partial explanation of the minimal injury after noxious stimulation.

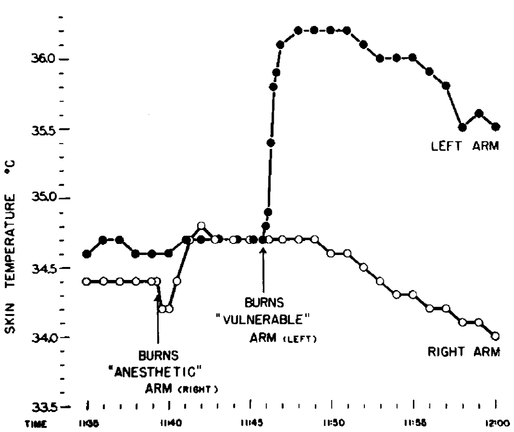


Fig. 14. Measurements during hypnosis of the skin temperatures of the right ("anesthetic") arm and the left ("vulnerable") arm. Previous to and during the application of burns (500 mc/sq. cm/sec. for three seconds) the right arm was suggested to be numb, anesthetic, and without feeling, whereas the left arm was suggested to have been damaged, to be very painful, and about to be even more painfully burned. Note the predictably greater degree and more persistent rise of skin temperature after suggestion of damage, indicating the pertinence of vasodilatation to the enhanced inflammatory reaction to noxious stimulation.

of the perfusate from this arm (Fig. 15), as compared with the "anesthetic" arm. The greater rise in skin temperature occurred in all experiments in which damage was greater on the "vulnerable" arm. A corresponding increase in finger pulsations was less predictable.

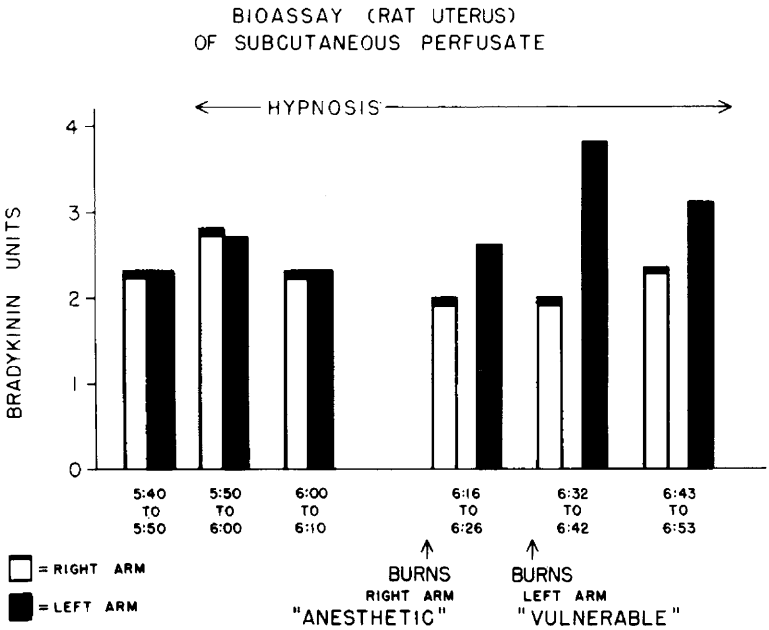
Comment:

Both the experiments with immersion in hot water, implicating the thermoregulatory centers in the brain stem and hypothalamus, and the experiments with hypnosis, implicating the highest levels of neural integration, demonstrate that activity within the central nervous system at several levels can augment the effects of the axon reflex. The occurrence of spontaneous urticaria observed in one susceptible subject suggests a widespread heightened reactivity in the vulnerable arm during the threatening situation created by suggestion and by burns.

It thus appears that the subject's perceptions and attitudes may be relevant to neural activities that engender or enhance inflammatory reactions. The liberation or accumulation of proteolytic enzymes in the periphery and the subsequent formation of a bradykinin-like humoral agent are implicated in this reaction.

Enhanced inflammation has been shown to be effective in combating invasion by microorganisms and in the rapid elimination of

Fig. 15.—Bioassay of the subcutaneous perfusate, expressed as units of a standard preparation of bradykinin inducing equivalent contractions of the rat uterus. After injury of the "vulnerable" arm during hypnosis there was more "bradykinin" activity in the perfusate from the flare zones induced by the thermal injury than in the perfusate from the arm suggested to be "anesthetic." Decimal points should precede numbers on left side.



tissue-breakdown products of injury. The view is proposed that man includes among his adaptive and protective devices neural reactions integrated at the highest levels that heighten inflammation in the peripheral tissues and increase the local susceptibility to injury, thus enhancing the protection of the whole organism at the cost of the integrity of a part. Such reaction at times may be essential to survival, but, if evoked inappropriately or excessively, may contribute to disease, since non-noxious stimulation becomes noxious and mildly damaging stimuli result in greater injury.

Summary and Conclusion

In the flare zone of the axon reflex during the initial phase of the period of vasodilatation, pain threshold was lowered, and tissue damage and the inflammatory response to noxious stimulation were enhanced. At this time there was liberated or accumulated in the skin and subsurface tissue fluids a pharmacodynamically active substance. This substance induced itching, local vasodilatation, and edema, lowered blood pressure, lowered the pain threshold, induced delayed and slow contraction of the rat uterus and guinea pig ileum, and induced relaxation of the rat duodenum. It deteriorated at room temperatures, was stabilized by boiling, and was destroyed by chymotrypsin. It was not acetylcholine, histamine, or serotonin, although these and other relevant agents may also have been present. It had many of the properties of a polypeptide of the bradykinin type.

During the initial phase of thermoregulatory-reflex vasodilatation in the arm stemming from immersion of the feet and legs in hot water, the pain threshold was lowered, and tissue damage and the inflammatory response to noxious stimulation were enhanced.

After standard amounts of noxious stimulation on the forearm during hypnosis, decreased inflammatory reaction and tissue damage were observed when the suggestion was made that the arm was insensitive and numb and would not be hurt, "anesthetic," as compared with the reaction and tissue damage of the other arm, which was suggested to be normally sensitive.

After standard amounts of noxious stimulation on the forearm during hypnosis, increased inflammatory reaction and tissue damage were observed in subjects who had received the suggestion that the forearm was tender, painful, and injured, "vulnerable," as compared with arms suggested to be normally sensitive or "anesthetic." Recordings of finger-pulse amplitude and skin temperature indicated that local vasodilatation following exposure to noxious stimulation was larger in magnitude, and persisted longer, in the "vulnerable" arm. The subcutaneous perfusate from the arm suggested to be painful, tender, and damaged developed a greater increase of bradykinin-like substance in response to standard noxious stimulation than did that from the arm suggested to be "anesthetic."

Neural activity involving the segmental or axon levels, the brain stem, and hypothalamic levels, as well as the subcortical and cortical levels, can alter the reactions in the peripheral tissues subserved in such a way as to augment inflammation and increase local tissue damage in reaction to noxious stimulation. Proteolytic enzymes and a bradykinin-like polypeptide are implicated in these enhanced reactions.

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